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Effect of Different Salinity on the Growth of *Chlorella* sp in Laboratory Scale Culture

Pengaruh Salinitas Berbeda terhadap Pertumbuhan *Chlorella* sp pada Kultur Skala Laboratorium

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ABSTRACT

This study aimed to determine the optimal salinity in increasing the growth rate of *Chlorella* sp in laboratory scale culture. This research was conducted in July 2023 at the Marine Biotechnology Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Riau. The experimental method was applied using a complete randomized design (CRD) with four treatments and three replicates. The treatments were as follows: T0 (control), T1 (25 ppt salinity), T2 (30 ppt salinity), and T3 (35 ppt salinity). The initial density of *Chlorella* sp was 250x10^3 cells/mL, and cell abundance observations were carried out for 14 days. The parameters observed were cell abundance, specific growth rate, and water quality (temperature and pH). The results showed that different salinity influenced the growth of *Chlorella* sp. 35 ppt salinity gave the best results on cell abundance of 266.66x10^4 cells/mL, with peak population occurring on day 10. Water quality during the study was still in the normal range and can be tolerated for the growth of *Chlorella* sp, and the temperature ranged from 25-31^{o}C and pH 6.4-8.0.

Keywords: Salinity, Cell abundance, *Chlorella* sp

ABSTRAK

Tujuan penelitian ini untuk mengetahui salinitas optimal dalam meningkatkan laju pertumbuhan *Chlorella* sp pada kultur skala laboratorium. Penelitian ini dilaksanakan pada bulan Juli 2023 di Laboratorium Bioteknologi Kelautan Fakultas Perikanan dan Kelautan, Universitas Riau. Metode yang digunakan adalah metode eksperimen, dengan menerapkan rancangan acak lengkap (RAL) dengan empat perlakuan dan tiga ulangan. Adapun perlakuannya sebagai berikut: T0 (kontrol), T1 (salinitas 25 ppt), T2 (salinitas 30 ppt), dan T3 (salinitas 35 ppt). Kepadatan awal sela *Chlorella* sp sebanyak 250 x10^3 sel/ml, pengamatan kelimpahan sel dilakukan selama 14 hari. Parameter yang diamati, yaitu kelimpahan sel, laju pertumbuhan spesifik dan kualitas air (suhu dan pH). Hasil penelitian menunjukkan bahwa perbedaan salinitas memberikan pengaruh terhadap pertumbuhan C*hlorella* sp. Salinitas 35 ppt memberikan hasil terbaik terhadap kelimpahan sel yaitu 266,66x10^4 sel/ml dengan puncak populasi terjadi pada hari ke-10. Kualitas air selama penelitian masih berada pada kisaran normal dan dapat ditoleransi bagi pertumbuhan *Chlorella* sp, yaitu suhu berkisar antara 25-31^{o}C dan pH 6,4-8,0.

Kata Kunci: Salinitas, Kelimpahan sel, *Chlorella* sp

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INTRODUCTION

Microalgae are microscopic organisms that grow through photosynthesis (Sani et al., 2014). Microalgae can grow quickly in the right climatic conditions (Aulia et al., 2021). In the field of aquaculture, it is used as a natural feed for fish larvae. In contrast, in the pharmaceutical industry, it is used as a food supplement with protein, lipids, and various minerals (Imelda et al., 2018). Indonesian waters have 1,500 species of microalgae spread in marine and fresh waters (Selviana et al., 2021) and have a variety of sizes, shapes, and types (Dayana et al., 2022). One type of microalgae that is commonly found in water areas and has the potential to be cultivated is *Chlorella* sp.

Chlorella sp is one of the microalgae that are used as a natural feed for freshwater and marine fish larvae such as tilapia (Yulita, 2015), bandeng (Supryady et al., 2022), grouper (Edy et al., 2022), white snapper (Astuti et al., 2023) as rotifer feed, shrimp (Andriani et al., 2023), and food supplements (Widyartini et al., 2022). According to Canelli et al. (2020), *Chlorella* sp contains carbohydrates ranging from 8.5-20.4%, protein 59.6-65.6%, fatty acids 8.4-11.2%, vitamins, minerals, chlorophyll, enzymes, and high fiber. According to Brahmantara et al. (2015), the growth rate of microalgae is influenced by N, P, K, C, H, Mg and Ca nutrient factors.

The utilization of microalgae in large quantities is often hindered by natural availability, so it is necessary to find ways to multiply microalgae by culturing. Factors that affect *Chlorella* sp's growth rate are nutrient availability and environmental conditions. On a laboratory scale, *Chlorella* sp culture generally uses practical fertilizers such as Guillard, Conway, Walne and agricultural fertilizers (Madalisa et al., 2022; Delilla *et al.*, 2022). External factors that affect *Chlorella* sp's growth are salinity (Lasmarito et al., 2022).

According to Djunaedi et al. (2017), *Chlorella* sp culture media salinity ranges from 25-35 ppt. Microalgae have a range of tolerance to certain salinities. Some microalgae species can live and develop well in low, medium, or high salinity ranges, while others can only survive in a specific range. Widyartini et al. (2022) stated that salinity changes also affect osmoregulation, nutrient availability, and competition with other organisms that impact the growth of *Chlorella* sp. High salinity will affect microalgae growth. Previous research has conducted different salinity concentrations on *Chlorella* sp microalgae (Aulia et al., 2021; Widyartini et al., 2022; Iba et al., 2019; Lasmarito et al., 2022).

Based on this background, it is necessary to culture *Chlorella* sp using different salinities to determine the cultivation that can be produced in large quantities. This study aimed to determine the optimal salinity in increasing the growth rate of *Chlorella* sp in laboratory scale culture.

MATERIALS AND METHOD

Time and place of research

The research was conducted in July 2023 at the Marine Biotechnology Laboratory, Faculty of Fisheries and Marine, Universitas Riau.

Research Method

The research method used is an experimental method, with the research design being a completely randomized design (CRD), with one factor, four treatments, and three replications. The dose of Walne used in this study was 1 mL/L. The treatments used are as follows:

T0: 0 ppt salinity (control)
T1: Salinity 25 ppt
T2: Salinity 30 ppt
T3: Salinity 35 ppt

Container Preparation

The culture containers used were 1.5 L volume bottles, 12 of which were washed, dried, and sterilized. After cleaning, each container was filled with 1 L of water. Salinity determination was carried out by diluting seawater until it reached the desired salinity (0, 25, 30, 35 ppt), and Walne fertilizer at a dose of 1 mL/L was put into all containers. Next, the containers were arranged randomly under the lamp, and aeration was adjusted.

Calculation of *Chlorella* sp

The initial density of *Chlorella* sp cells was 250 x10^3 cells/mL and cell abundance was calculated daily for 14 days. The counting procedure refers to the research of Effendi et al. (2023), where *Chlorella* sp was

observed using a hand counter and a hemocytometer. Calculate the density of *Chlorella* sp with a Haemocytometer, which takes *Chlorella* sp in each container using a drop pipette and drips it on the transverse part of the trench until complete. Dripping is done carefully to avoid air bubbles under the cover glass. Furthermore, the Haemocytometer was observed under a microscope with a magnification of 10x40. To determine the density of *Chlorella* sp, the cells are counted in a square box with a side of 1 mm and used on a hand counter.

Water Quality Measurement

Water quality measurements were taken at the beginning, middle, and end. The measured water quality includes pH and temperature.

Cell Density

The number of microalga cells can be calculated with the cell abundance formula according to Armanda (2013) as follows:

$$\text{Cell density (cell/mL)} = \frac{\text{Number of cells in field of view (n)}}{\text{Number of fields of view (5)}} \text{ x } 25 \text{ x } 10^4$$

Specific Growth Rate

The specific growth rate is the speed of growth in the population in a certain unit of time, the specific growth rate with the formula (Wood et al., 2005).

$$\mu = \frac{\text{Ln X2} - \text{Ln X1}}{t2 - t1}$$

Description:

μ	: Specific growth rate (cell/day)	t1	: Initial sampling time (days)
X1	: Initial cell density (cells/mL)	t2	: End of sampling time (days)
X2	: Final cell density (cells/mL)		

RESULT AND DISCUSSION

Cell Abundance of *Chlorella* sp

Data obtained during the study on the abundance of *Chlorella* sp cultured using different salinities in each treatment are presented in Table 1.

Table 1. Average abundance of *Chlorella* sp cultured with different salinities

Observation time (days)	*Chlorella* sp abundance			
	T0 (Control)	T1 (25 ppt salinity)	T2 (30 ppt salinity)	T3 (35 ppt salinity)
1	250.000±86.602	233.333±28.868	200.000±50000	183.333±57735
2	300.000±50.000	266.667±76.376	233.333±57735	216.667±28.868
3	383.333±28.868	350.000±86.602	300.000±500.00	283.333±28.868
4	500.000±50.000^{b}	466.667±28.868^{ab}	400.000±500.00^{a}	383.333±28.868^{a}
5	666.667±28.868^{b}	650.000±50.000^{b}	566.667±28.868^{a}	550.000±50.000^{a}
6	866.667±28.868	866.667±76.376	783.333±57.735	733.333±28.868
7	1.050.000±50.000^{ab}	1.200.000±50.000^{b}	1.050.000±50.000^{a}	1.033.333±57.735^{a}
8	1.316.667±57.735^{a}	1.516.667±76.376^{b}	1.433.333±57.735^{ab}	1.433.333±28.868^{ab}
9	1.533.333±104.083^{a}	1.800.000±50.000^{b}	1.800.000±86.602^{b}	1.783.333±76.376^{b}
10	1.800.000±100.000^{a}	2.100.000±50.000^{b}	2.116.667±28.868^{b}	2.166.667±28.868^{b}
11	1.633.333±28.868^{a}	1.816.667±125.830^{b}	1.833.333±28.868^{b}	1.866.667±76.376^{b}
12	1.566.667±28.868^{a}	1.733.333±28.867^{b}	1.766.667±28.868^{b}	1.783.333±76.376^{b}
13	1.450.000±86.602	1.600.000±200.000	1.666.667±28.868	1.700.000±50.000
14	1.250.000±50.000^{a}	1.350.000±50.000^{ab}	1.483.333±144.338^{b}	1.516.667±57735^{b}

Description: Mean values in the same column followed by the same letter indicate not significantly different (p>0.05).

Table 1 shows that all treatments experienced increased growth until the exponential phase. The highest abundance of *Chlorella* sp. cultured using different salinity occurred on day 10, which ranged from 180.00 - 216.66 x 10^4 cells/mL. Growth of *Chlorella* sp at 35 ppt salinity can produce the highest abundance on day 10, 216.66 x10^4 cells/mL. The lowest salinity at 25 ppt is 210.00x10^4 cells/mL. Furthermore, in the control

treatment, the abundance of *Chlorella* sp was 180.00 x 10^4 cells/mL. The analysis of variance (ANOVA) test showed that the abundance of *Chlorella* sp cells cultured using different salinity gives a natural effect ($p<0.05$).

Based on Figure 1, it can be seen that P3 (35 ppt salinity) can produce the highest abundance of *Chlorella* sp cells, which is 266.66x10^4 cells/ml. This is thought to be due to the optimum salinity of 35 ppt for the growth of *Chlorella* sp, which affects osmotic pressure in absorbing nutrients. Supported by Lasmarito et al. (2022), optimal salinity will maintain the balance of osmotic pressure between microbial cells and culture media so that the growth and development of microalgae will be optimal. Then Mata et al. (2010) added that changes in salinity affect microalgae in three ways: osmotic pressure, ion pressure, and ionic ratios due to membrane permeability. Kusumaningrum & Zainuri (2013) stated that in high salinity, microalgae cells can survive because of the help of glycerol, which functions as a supporter of osmotic pressure to balance the osmolarity process in the outer cell (extracellular).

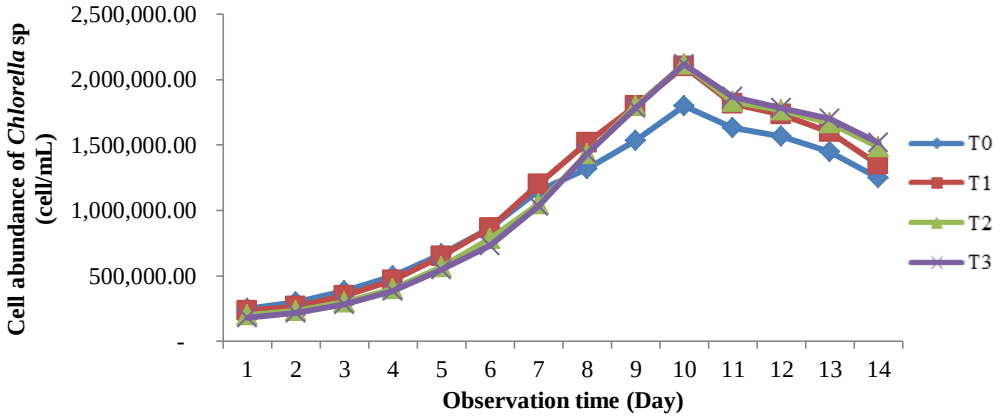

Figure 1. Cell abundance of *Chlorella* sp with Different Salinity

Salinity is one of the factors that affect lutein content in microalgae. Stress conditions in *C. vulgaris* will produce carotenoid pigments that protect chloroplasts from oxidative damage, resulting in high carotenoid pigments (Lasmarito et al., 2022). Carotenoid pigments are used not in the growth process but as self-defence when microalgae experience stress (Kimberly et al., 2019).

In addition, an essential factor in the growth of *Chlorella* sp. is nutrients in the culture medium. Hakalin et al. (2014) stated that nutrients are used for cell division and metabolic processes. If nutrients such as nitrogen (N) and phosphorus (P) are depleted or limited in the media, it will reduce the rate of production or division of microalgae. The nutrients used in this study are Walne, which has a complex micronutrient content such as N, P, Fe, Cu, Co, and Zn (Triastuti et al., 2011) so that *Chlorella* sp can grow well. A change in the color of the culture medium usually indicates a significant increase in cell abundance during the growth phase. Before cell growth occurs, the media's color is clear, and the culture colour turns greenish after cell division. This is supported by Sintya (2018), who states that during microalgae cultivation, the color of the culture changes at each growth phase.

The control media (T0) produced an abundance of *Chlorella* sp at 148.33 x 10^4 cells/mL, lower than the other treatments. It is suspected that Chlorella sp. does not experience osmotic pressure disorders, so it is less optimum in producing lutein pigments. While salinity 35 has a high abundance, so the carotenoid content also increases. This is also supported by Agustini (2014), which states that the amount of carotenoids is proportional to the abundance of Chlorella. The higher the abundance of microalgae, the higher the carotenoid content. The higher the carotenoid content, the higher the lutein content. Then, Aulia et al. (2021) also stated that low salinity will cause microalgae cells to experience a hypotonic state, where the condition of the solution in the environment has a lower concentration than the liquid in microalgae cells (cells will expand).

Specific Growth Rate of *Chlorella* sp

Chlorella sp consists of adaptation, exponential, stationary, and death phases. The adaptation phase in this study lasted, on average, within two days, namely the observation of days 0 to 2, and the cell density continued to increase. *Chlorella* sp specific growth rate graph cultured with different salinity can be seen in Figure 2.

The exponential phase is a phase of cell division where the occurrence of very rapid growth evidences this phase. According to Wibowo et al. (2017), the exponential phase begins with cell division with a continuous

growth rate. The exponential phase occurs until cell density reaches maximum density. In this study, there was an increase in cell abundance on day one until the peak population on day 10. The increase in the growth rate value from the adaptation phase to the exponential phase occurs because the cell density continues to increase daily. Cell growth is directly proportional to the specific growth rate because optimal cell growth will produce an optimal specific growth rate (Sigalingging et al., 2019).

The magnitude of the growth rate in treatment T3 (salinity 35 ppt) is thought to be due to optimal salinity so that the growth of Chlorella increases. Djunaedi et al. (2017) supported the idea that *Chlorella* sp can grow at 35 ppt salinity and was supported by nutrient factors in the culture medium. The growth rate decreased starting on day 11 until day 14. Cell abundance tends to remain in the stationary phase because the number of dividing cells is equal to the number of dead cells. Another factor affecting the decrease in cell abundance is the content of nutrients in the culture medium (Zainuddin et al., 2017). The high nutrient content in the early phase of culture is used by microalgae cells to perform growth until the exponential phase. However, the absence of additional nutrients after the exponential phase can cause cell density to decline due to competition for existing nutrients (Kimberly et al., 2019).

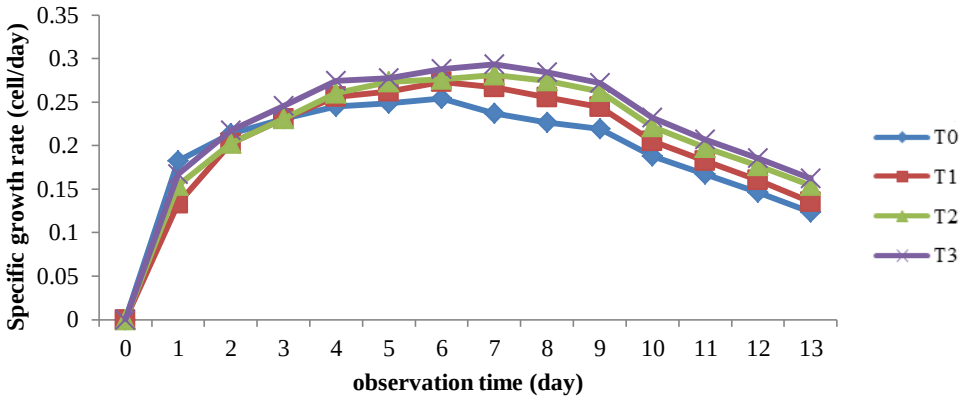

Figure 2. The specific growth rate of *Chlorella* sp

Furthermore, the death phase decreases the number of cultured organisms after passing the stationary phase. A higher death rate characterizes this phase than the production rate. Rosahdi et al. (2015) stated that the death phase occurs due to changes in water quality that are getting worse, reduced nutrients in the culture medium, and the inability of cells to metabolize.

Water Quality

Factors that affect microalgae growth include temperature and pH. Observation data of water quality parameters during the study are presented in Table 2.

Table 2. Range of values for water quality parameters

No.	Parameters	Treatment			
		T0	T1	T2	T3
1	Temperature (^{o}C)	26.5 - 30	25 - 31	26,5-30	26-30
2	pH	6.5 – 8.0	6.5-7.8	6.6-7.7	6.4-7.6

The results of temperature measurements on *Chlorella* sp culture media ranged from 25-31^{o}C. This temperature range is still good for the growth of *Chlorella* sp. The temperature range that can still be tolerated for the growth of *Chlorella* sp is 25-35^{o}C (Budiardi et al., 2010) and 20-35^{o}C (Mookiah et al., 2020). The degree of acidity (pH) has a vital role in the growth of the cell population of *Chlorella* sp. In this study, the pH obtained ranged from 6.4 to 8. This pH range can still be tolerated for the growth of *Chlorella* sp. According to Mookiah et al. (2020), *Chlorella* sp can grow on media with a pH of 4-8.

CONCLUSION

Based on the study's results, it can be concluded that *Chlorella* sp at 35 ppt salinity can produce cells with an abundance reaching 216.66 x 10^{4} cells/mL.

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